university of groningen

University Medical Center Groningen

University of Groningen

The extracellular proteinase of Lactococcus lactis

Laan, Harm Willem Frederik

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1991

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Laan, H. W. F. (1991). The extracellular proteinase of Lactococcus lactis. Groningen: s.n.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 12-11-2019

SUMMARY

Lactococci are used in the production of fermentated dairy products, of which cheese is one of the most important. In starter cultures used in dutch cheese manufacturing *Lactococcus lactis* is the dominant species. The main functions of the Lactococci are a fast conversion of lactose into lactate and the formation of flavour compounds. For both functions proteolytic enzymes are of crucial importance. For a rapid lactate formation rapid growth of these auxotrophic organisms is required, which in milk depends on the availability of small peptides and free amino acids. In milk these medium components are present at low concentrations, and can be formed from casein due to the activity of the proteolytic enzymes, proteinases and peptidases. Casein degradation by *L. lactis* is initiated by the extracellular serine proteinase, which is essential for growth in milk. In the first chapter the characteristics of the *L. lactis* proteinase are reviewed. Proteinases of different *L. lactis* are very similar. This cell envelope-associated enzyme is primarily synthesized as pre-pro-proteinase and is 200 kilodaltons. After biochemical purification a lower molecular weight is always observed (between 80 and 145 kDa). An immunological analysis identified proteinase components which are indicated as A and B for the proteinase of *L. lactis* subsp. *cremoris* WG2 these components are indicated as A and B.

In chapter 2 the isolation of six types of monoclonal antibodies is described, which are directed to the components A or B of the proteinase of *L. lactis* subsp. *cremoris* WG2. The reactions of the monoclonal antibodies with the proteinases of different *L. lactis* strains again reveal that these enzymes are very similar. They can be distinguished by small differences in their amino acid sequences. Two classes of proteinases with different substrate specificities can be distinguished by the isolated monoclonal antibodies. In chapter 3 two regions are localized in the proteinase molecule to which the monoclonals bind. Each region represents one of the components. The antigenic determinants of component B become accessible only when the proteinase is (auto)proteolytically active. Component A and B can be detected in a single proteinase molecule only after denaturation. From these data it is concluded that the proteinase components A and B are different conformations of the enzyme.

In chapter 4 it is demonstrated that the *L.lactis* proteinase is released from the cell envelope due to self digestion. The release is inhibited by specific proteinase inhibitors, as well as in the presence of Ca^{2+}. In a model this inhibition is explained by the stabilisation of an enzyme conformation by Ca^{2+}. In this conformation self digestion sites are masked and the proteinase remains attached to the cell. Amino acid analysis revealed that self digestion occurs at the C-terminus by cleaving of the membrane anchor sequence. Deletion of this sequence results in the release of the proteinase.

The autodegradation of the released proteinase is studied in chapter 5. This occurs during purification of the enzyme. The low molecular weight values that have been found after purification, can be explained by self digestion. Ultimate-

ly, self digestion leads to inactivation of the proteinase by deletion of the active site region or possibly by deletion of a region important for the binding of substrate.

In chapter 6 the regulation of the production of the *L. lactis* proteinase under different growth conditions is studied. The organisms were grown in a chemically defined medium to accurately control all parameters. It appears that both the nitrogen source and the growth rate are important factors in the regulation of proteinase production. In the presence of casein or peptides as the sole source of nitrogen, the proteinase production is low, while in the presence of amino acids the production is higher. During growth in a medium containing amino acids as well as casein the proteinase production is the same as in the presence of casein only. These results indicate that peptides which are released after casein hydrolysis, inhibit proteinase synthesis. The exact mechanism of the regulation of proteinase synthesis has to be further elucidated.

The use of monoclonal antibodies has been shown to be very valuable in the characterisation of the *L. lactis* proteinase. In crude preparations as well as in the culture medium the enzyme (or enzyme fragments) can be detected specifically and can be quantitated. It is interesting that different conformations of the proteinase of *L. lactis* can be detected only by immunochemical techniques.

SAMENVATTING

Lactoc
produkte
gebruikt
belangrij
omzettin
Voor bei
snelle m
die in me
aminozu
ties aanv
de aktivit
wordt ge
voor de g
van de ei
verschille
envelop g
en is 200
gewicht g
giese ana
lactis sub
duid.

In hoofc
ven, die g
subsp. *cr*
van versc
deze enz
de amino
specificite
antilichan
gelokalise
tatief voo
van komp
aktief is. I
aangetoo
dat de pro
molekuul
In hoofd
van het *L*
tie wordt
aanwezig
stabilisati
konforma
aan de c
hydrolyse